Effect of 2-thiouracil on the infectivity of tobacco mosaic virus

Among compounds which are known to inhibit the multiplication of tobacco mosaic virus, 2-thiouracil is one of the most effective[1,2]. When the virus multiplies in leaves supplied with 2-thiouracil the analogue is incorporated into the virus RNA[3,4]. No difference in infectivity of the virus from normal and thiouracil-treated leaves was detected[1,2], although the question was not studied in detail. JEENER[5] confirmed these results being unable to find a difference between normal virus and that containing 2-thiouracil in their infectivity towards *Nicotiana glutinosa*. On the other hand, he found that virus containing the analogue had a slower rate of multiplication in *N. tabaccum*. These findings led him to speculations regarding the structure of the tobacco mosaic virus particle[5]. We have re-investigated the effects of 2-thiouracil on the infectivity of the virus, and conclude that when the latter is grown in the presence of the analogue it is substantially less infectious than normal virus.

Leaves of *N. tabaccum* var. *White Burley*, were inoculated with a 1:5 dilution of sap from tobacco leaves infected with tobacco mosaic virus. The inoculated plants were kept under continuous illumination from fluorescent light for 24 h, after which the leaves were excised, divided by cutting away the mid-rib and the two sets of half leaves floated on Vickery's solution adjusted to pH 6.4, one set with and one without 2-thiouracil. After a further 8 days the leaves were harvested, washed and frozen. The material was then ground, thawed and the sap extracted, heated at 55° for 5 min and centrifuged for 10 min at 2,500 rev./min. Relative infectivities of the sap from control and thiouracil-treated leaves was assayed by inoculating a series of 2-fold dilutions to half leaves of *N. glutinosa*. We determined experimentally that the maximum possible concentration of 2-thiouracil in such inocula was less than 0.01 times the minimum amount required to affect local lesion production in *N. glutinosa*.

Total virus material in the clarified sap was determined in most experiments by the serological-chromatographic method[6] which gives an estimate of the amount of virus RNA associated with the virus protein antigen. In some experiments the amount of virus was estimated from total N determinations on virus precipitated by antiserum in the region of antibody excess or the equivalence zone. Virus N was calculated by reference to a standard curve for the antiserum. In other experiments the number and length of virus particles in diluted sap was estimated by counts from electron micrographs using the spray drop method and polystyrene latex spheres as a reference particle[7,8]. There was no significant difference between control and thiouracil virus in the size distribution of rods with a width of 15 mμ.

The reduction in yield of virus by thiouracil was considerably greater when estimated by infectivity tests than when the total virus material present in the sap was determined by any of the three methods used (Table I). These data suggest that 56–80 % of the virus particles in the thiouracil samples were rendered non-infectious.

We have also carried out infectivity tests on partially purified virus preparations made by ultra-centrifugation and by salt precipitation. With such preparations the apparent reduction in infectivity by thiouracil was lower. For example, in a typical experiment it was calculated from data on tests with clarified sap that 70 % of the thiouracil virus was rendered non-infectious. Tests with partially purified prepa-

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

TABLE I

EFFECT OF 2-THIOURACIL ON YIELD OF TOBACCO MOSAIC VIRUS FROM TOBACCO LEAVES ESTIMATED BY VARIOUS METHODS

Expt.	Yield of thiouracil virus in clarified sap expressed as a percentage of control virus estimated by:			
	Relative infectivity	Virus RNA	Virus N	Electron Microscopy*
1	8	48		40
2	< 8	29		36
3	8	28		31
4	19	–	52	–
5	26	–	59	–

* Percentages based on total length of rod-shaped particles with a width of 15 mμ.

rations, however, indicated that only 37 % had been made incapable of producing lesions. It is well known that procedures such as centrifugation and salt or pH precipitation used to purify tobacco mosaic virus lead to some end to end aggregation of the virus rods. The reduced effect of the virus we have observed with purified preparations could be caused by such aggregation without assuming any difference in degree of aggregation, between normal and thiouracil virus. It may also account, at least in part, for the fact that JEENER[5] could find no difference in the infectivity for *N. glutinosa* of normal virus and virus containing thiouracil. The incorporation of 8-azaguanine into the RNA of tobacco mosaic virus[6] and of 5-bromouracil into the DNA of T_2 phage[9] can lead to the production of sterile virus. From our results we conclude that the incorporation of 2-thiouracil into the RNA of tobacco mosaic virus has a similar effect.

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